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Occurrence of *Phytophthora cinnamomi* in the Rhizosphere of Declining *Leucadendron elimense* subsp. *salteri* in South Africa

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ABSTRACT

Phytophthora cinnamomi is a destructive invasive plant pathogen reported from more than 5000 hosts and across all continents except Antarctica. In South Africa, *P. cinnamomi* poses a substantial threat to native flora, particularly members of the Proteaceae. This pathogen has been detected in diverse settings on hosts within this family, and, of especial concern, in natural populations of species under a high threat of extinction. In this study, *P. cinnamomi* was detected in a population of *Leucadendron elimense* subsp. *salteri* located in a nature reserve. This plant population has experienced a severe decline in recent years. Two plants collected for laboratory analysis exhibited severe collar lesions and had lost most their root system due to root rot, fitting with symptoms typically associated with *P. cinnamomi* infection in susceptible hosts. Samples were processed, and the presence of *P. cinnamomi* was confirmed morphologically and molecularly from the rhizosphere soil of both plants. There is an urgent need to mitigate the impacts of *P. cinnamomi* in South Africa, in particular by expediting the regulatory evaluation of potential treatments.

1 | Introduction

Phytophthora cinnamomi is widely regarded as one of the most damaging invasive plant pathogens in the world. With over five thousand known host species and a distribution spanning at least 78 countries (Hardham and Blackman 2018), this pathogen is included in the Global Invasive Species Database list of 100 of the world's worst invasive alien species (https://www.iucngisd.org/gisd/100_worst.php).

In South Africa, *P. cinnamomi* was first detected in 1931 from avocado (*Persea americana*). It has since been reported from a wide range of commercial crops and native plant species. There are 140 confirmed host species in South Africa (see supplementary material to Paap et al. 2025), of which 101 are native and 93 endemic. A diverse range of these species is considered highly susceptible, highlighting the potential impact of *P. cinnamomi* if introduced into their native ranges.

The threat posed by *P. cinnamomi* to South African flora is particularly serious for plants in the Proteaceae (Paap et al. 2025). South Africa represents a major regional centre of diversity for the family, with 357 native species currently recognised, the majority of which occur in the Cape Floristic Region (CFR; Figure 1). Many of these species are range-restricted endemics and face an elevated risk of extinction due to land-use change, habitat degradation, invasive alien plants, altered fire regimes and climate change (Skowno et al. 2021). The presence of the invasive plant pathogen *P. cinnamomi* constitutes an additional and significant driver of decline; however, its contribution to

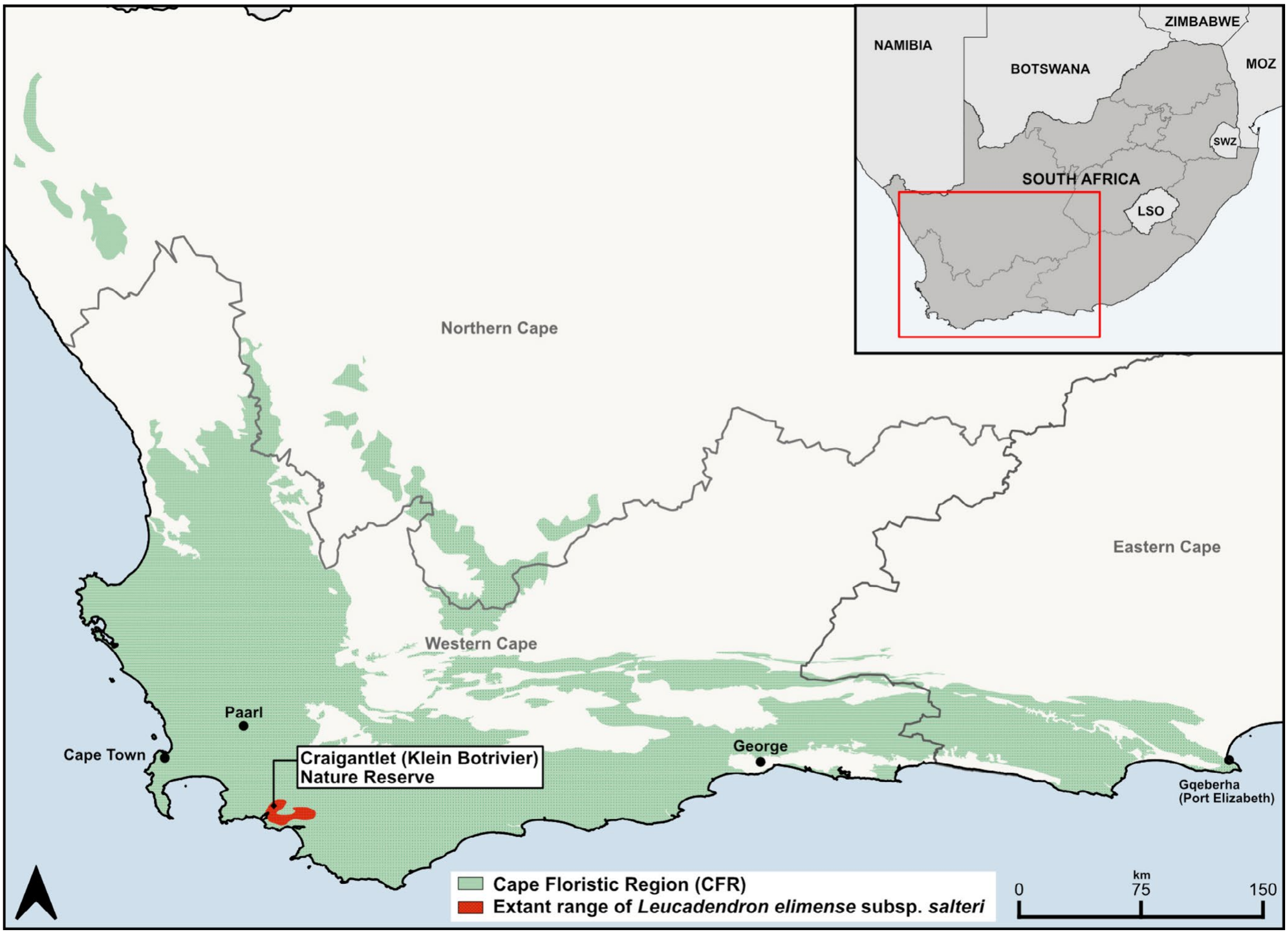

FIGURE 1 | Geographical distribution of the Cape Floristic Region (CFR), and current extant distribution of *Leucadendron elimense* subsp. *salteri*.

extinction risk is rarely explicitly considered in conservation assessments (Paap et al. 2025).

In this study, we investigated a decline occurring in a population of *Leucadendron elimense* subsp. *salteri* (Caledon conebush), an endemic Proteaceae found in the western Overberg region of the Western Cape, South Africa. The taxon has lost more than 70% of its original habitat due to crop cultivation and mining, and the remaining sub-populations are confined to a highly restricted geographic range, with an extent of occurrence of 203 km^2 and an area of occupancy of just 36 km^2 (Figure 1). Only four isolated subpopulations persist, most of which occur in fragmented habitats between agricultural lands. Population sizes at all known locations continue to decrease due to alien plant invasion, ongoing habitat loss to agriculture and wildflower harvesting. *Leucadendron elimense* subsp. *salteri* is currently classified as Endangered under the IUCN criteria (Rebelo et al. 2020).

A population of *L. elimense* subsp. *salteri* at Craigantlet (Klein Botrivier) Nature Reserve, Botrivier, Western Cape has been declining over the preceding 3 years (2021–2024). In July 2025, conservation staff from the CapeNature Overberg Landscape Unit noted extensive decline and symptoms of yellowing, wilting, dieback and mortality (Figure 2). At this time, approximately 75% of plants exhibited visible symptoms, with all age classes affected (T. Mabena, *pers. comm.*). Given the rapid deterioration and the restricted extent of the population, symptomatic plants and associated soil were sampled for diagnosis.

2 | Materials and Methods

In early September 2025, rhizosphere soil and collar material were collected from two symptomatic plants (GPS coordinates: −34.270310, 19.178124) and submitted to the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Samples were examined and processed within 2 weeks of collection. Visual inspection of the samples revealed extensive discolouration of the root collars and primary roots (Figure 2c). Secondary and fine roots present in the soil exhibited pronounced discolouration or were completely necrotic. Prior to isolations on culture medium, collar tissues were surface disinfested with 70% ethanol, after which small segments were excised and plated onto *Phytophthora*-selective medium, NARP (50 mg nystatin, 200 mg ampicillin, 10 mg rifampicin and 25 mg pentachloronitrobenzene per 1 L distilled water and 17 g cornmeal agar). Rhizosphere soil samples were baited for *Phytophthora* following the methods outlined by Paap et al. (2023). Plates were incubated at 22°C and examined daily using microscopy for growth typically characteristic of *P. cinnamomi* (coralloid hyphae with hyphal swellings). Single hyphal tip isolations of putative *Phytophthora* isolates were

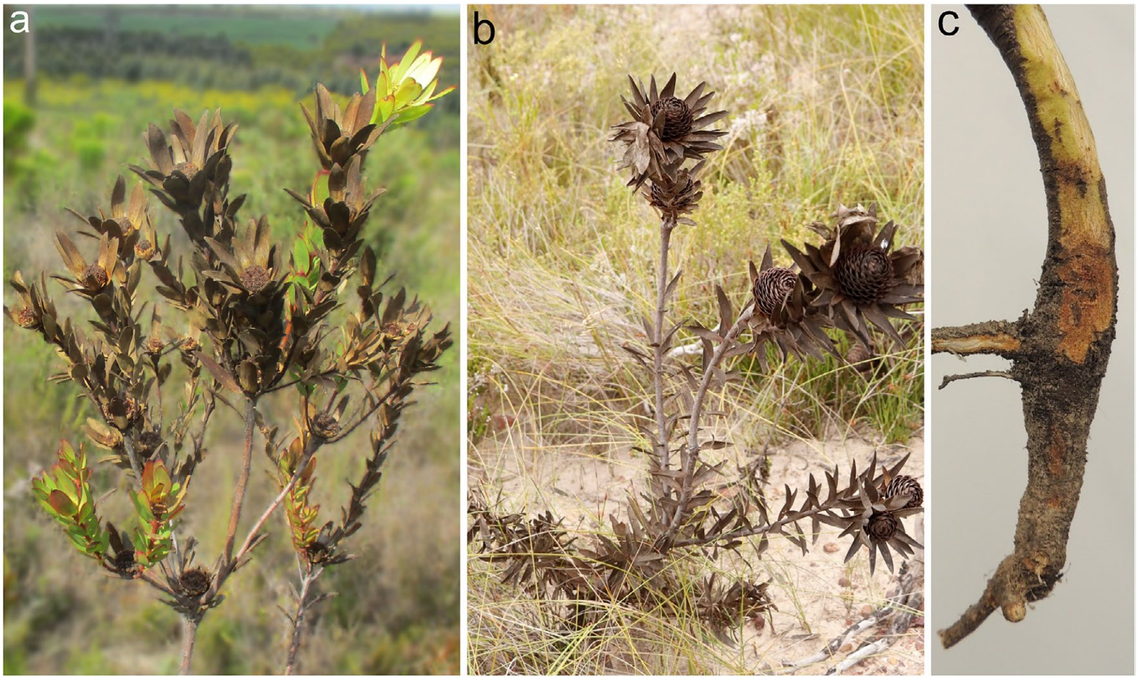

FIGURE 2 | Decline symptoms on *Leucadendron elimense* subsp. *salteri*. (a, b) Symptoms observed at Craigantlet (Klein Botrivier) Nature Reserve, Botrivier. (c) Root collar lesions observed in laboratory inspections. Photo credits: (a, b) Thandeka Mabena.

prepared using a dissection microscope and transferred onto fresh half strength potato dextrose agar (½PDA; 19.5 g PDA powder, Merck, South Africa, 7 g Difco agar, 1 L distilled water).

A representative isolate from each of the two *L. elimense* subsp. *salteri* samples was selected for DNA sequencing. DNA was extracted from seven-day-old cultures grown on ½PDA using PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific, USA), following the manufacturer's instructions. The internal transcribed spacer (ITS) was amplified using primers ITS6/ITS4. Amplicons were purified using ExoSAP-IT (Thermo Fisher Scientific, USA) and sequenced bidirectionally using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Thermo Fisher Scientific). Sequence assembly and trimming were performed in CLC Main Workbench v.8.0.1 (QIAGEN). Consensus sequences were compared against the *P. cinnamomi* ex-type (CPHST BL 12) reference sequence (MG865473).

3 | Results and Discussion

Phytophthora was not recovered from directly plated collar tissues. However, after 48–72 h of incubation, all leaf baits developed necrotic lesions, and *Phytophthora* colonies were obtained from all bait isolation plates. The morphology of isolates recovered from baited root and rhizosphere samples was consistent with that described for *P. cinnamomi*. Two isolates, one from each of the sampled plants, were purified and deposited in the culture collection (CMW) of FABI. These isolates (CMW68299 and CMW68300) were subsequently identified by DNA sequencing.

Amplification of the ITS region yielded products of approximately 720 bp. Both isolates produced identical sequences (GenBank accession numbers PZ414231 and PZ414232), which matched the ex-type reference sequence of *P. cinnamomi* (CPHST BL 12). Molecular data were therefore consistent with morphological identification of the isolates as *P. cinnamomi*.

Pathogenicity testing could not be undertaken due to the Endangered status of *L. elimense* subsp. *salteri*, and the absence of cultivated plants available for experimental inoculation. In addition, isolations from direct plated collar tissues were unsuccessful, likely due to tissue desiccation during the interval between collection, transport and processing. Although only two symptomatic plants were sampled, *P. cinnamomi* was consistently recovered from associated rhizosphere soil, and affected plants exhibited yellowing, wilting, root and collar rot, dieback and mortality. These symptoms are consistent with those commonly associated with *P. cinnamomi* infection in members of the Proteaceae (e.g., *Leucadendron argenteum*; Msweli et al. 2025, Paap et al. 2025). Although, Koch's postulates could not be fulfilled, the characteristic field symptoms observed, together with the well-documented susceptibility of Proteaceae members, particularly *Leucadendron* and *Leucospermum* species, to *P. cinnamomi*, strongly suggest that the presence of this pathogen is associated with the observed symptoms. Nevertheless, additional sampling of declining individuals in the field would help strengthen this association and provide further insight into the incidence and spread of the pathogen.

This study represents the second documented case in which the detection of *P. cinnamomi* has been associated with the decline of a threatened, range-restricted Proteaceae species in the CFR. Previously, Paap et al. (2023) reported extensive mortality of the Critically Endangered *Sorocephalus imbricatus*, with *P. cinnamomi* implicated as the likely causal agent. The present findings in *L. elimense* subsp. *salteri* reveal a similar pattern, characterised by rapid population decline, widespread root and collar rot and recovery of *P. cinnamomi* from affected sites, despite the absence of prior recognition of this pathogen as a threatening process in Red List assessments.

These cases highlight the concerning and likely under-recognised role of *P. cinnamomi* in driving declines of threatened Proteaceae in the CFR. Given the high proportion of rare, endemic and range-restricted taxa in the region, these observations underscore the urgent need for a coordinated research and management programme to assess pathogen distribution, host susceptibility and extinction risk. As outlined by Paap et al. (2025), such a programme is essential to inform conservation planning, guide mitigation efforts and ensure that invasive pathogens are appropriately incorporated into threat assessments and management strategies for CFR flora.

As with the decline of *S. imbricatus*, phosphite treatment may offer a practical means of maintaining threatened Proteaceae populations in situ. This could be either as a long-term protective measure or as an interim strategy to preserve individuals until reproductive maturity, allowing the collection of germplasm. Recent experimental work on South African Proteaceae supports this potential, with glasshouse trials demonstrating that phosphite can significantly reduce disease severity and mortality caused by *P. cinnamomi* in susceptible taxa (Msweli et al. 2025). However, despite demonstrated efficacy against *Phytophthora* species across a range of production systems and natural ecosystems, phosphite use in conservation settings can be constrained by regulatory frameworks governing agricultural remedies. Such regulatory limitations have been reported internationally, including in forestry and natural ecosystems, where proven treatments have been delayed or prohibited due to differences in product classification and authorisation (López-García et al. 2024). In situations such as those described here, where populations are small, declines are rapid, and time to intervene is limited, greater regulatory flexibility, through mechanisms such as conservation-specific or emergency use provisions, would enable timely intervention and may be critical to preventing further losses of highly threatened taxa in biodiversity hotspots such as the Cape Floristic Region.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Sharing of data generated in this study has been approved by CapeNature through a data sharing agreement approved in June 2026.

Peer Review

For transparency, the peer review documents associated with this article are available at https://doi.org/10.1111/efp.70097.

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